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ORIGINAL ARTICLE

Monitoring aspirin therapy in children after interventional cardiac catheterization: laboratory measures, dose response, and clinical outcomes

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Conclusion: While there was poor agreement among assays, AA and urinary 11-dhTxB2 show good specificity for the monitoring of aspirin therapy in children. Aspirin at 2–5 mg/kg inhibits platelet function; AR in children is rare and can be overcome by dose increase.

Keywords Platelets · Cardiac catheterization · Thromboembolism · Bleeding events · Aspirin dosing · Aspirin resistance

Abbreviations

AA	Arachidonate-induced aggregation
AR	Aspirin resistance
ICC	Interventional cardiac catheterization
IR	Interquartile range
PFA-100 CT	Platelet function analyser-100 closure time
Urinary 11-dhTxB2	Urinary 11-dehydro-thromboxane B_2

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VWD	von Willebrand disease
VWF	von Willebrand factor
VWF:RCo	VWF:ristocetin cofactor

Introduction

In children, aspirin is the most commonly used anti-platelet agent for the prevention of arterial thrombotic events. It is commonly used for the prevention of stroke recurrence and prophylactically after interventional cardiac catheterization (ICC) and cardiac surgery [3, 6, 16, 23, 26, 35, 36].

Aspirin is usually administered at a dose ranging from 2 to 10 mg/kg/day; higher aspirin doses are only used as anti-inflammatory treatment for children with Kawasaki disease [26, 35]. Still, a relatively small number of clinical trials assessing the monitoring and efficacy of aspirin have been conducted in pediatric patients. Current protocols for anti-thrombotic prophylaxis for ICC in children have been developed according to protocols from adults [22, 23, 26, 36]. In adults after ICC, thrombus formation on atrial septal defect and patent foramen ovale closure devices has been reported in 0–7 % of patients and differed between devices [19].

In the past, studies on both adults and children have reported a variability in aspirin response called aspirin "resistance" (AR). While this has led to concern among patients and physicians about the efficacy of aspirin, there is ongoing discussion as to the mechanisms responsible for the variability in anti-platelet effects and the role of different laboratory assays in the monitoring of aspirin therapy. While a failure of aspirin to produce a response on laboratory measures has been reported in 5–56 % of adult cases, depending on the clinical setting and the laboratory method used, an inability of aspirin to protect individuals from arterial thrombosis is found at a lower frequency of 4–10 % [7, 11, 12]. The poor correlation of different platelet function tests and the prediction of a thrombotic risk by laboratory measures of AR has been debated in the literature [5, 7, 10, 13, 15, 24, 30].

So far, four pediatric studies (one published in abstract form) have investigated AR; in each, children receiving aspirin for a variety of indications (e.g., stroke, cardiac surgery, and thrombocytosis) were included. For the determination of aspirin responsiveness, mostly global platelet function or point-of-care tests, i.e., the platelet function analyzer PFA-100® or the VerifyNow Aspirin® system, and urinary 11-dehydro-thromboxane B_2 (urinary 11-dTxB2) level measurements were used. An AR prevalence of 2.3–26 % was reported and was highly assay dependent. Again, only one study described children with thrombotic events; however, the authors were unable to correlate this with the laboratory response to aspirin [6, 16, 29, 40].

In view of the incomplete and limited amount of data available, the aims of this study were (1) to evaluate the usefulness and assay agreement of different laboratory tests for the determination of aspirin response, (2) to determine the prevalence of AR, and (3) to provide preliminary information about efficacy and safety of aspirin therapy, in a well-defined cohort of pediatric patients undergoing interventional cardiac catheterization.

Patients and methods

This prospective study was performed at the University Children's Hospital in Zurich, Switzerland, and was approved by the regional Ethics Committee. Written informed consent was obtained from patients and/or parents as appropriate; enrollment was between August 2006 and October 2010. Children with congenital heart defects undergoing ICC with dilatation of single valve stenosis, stent implantation or dilatation of isolated pulmonary artery or aortic stenosis, or device closure of atrial or ventricular septal defects or patent arterial duct, and requiring aspirin therapy for 3–6 months were included. Children were excluded from the study if they had one of the following conditions: a complex or univentricular heart malformation; dysmorphic syndrome interfering with platelet function or number, such as Noonan and thrombocytopenia-absent radius syndrome; known congenital metabolic or hemorrhagic disorders; known anemia (hemoglobin<100 g/l) and/or thrombocytopenia (platelet$<150\times10^9$/L); known aspirin allergy; intake of other medications interfering with platelet function; non-compliance; and parents unwilling to consent to the study. A detailed bleeding history was taken in all study subjects, and children with a positive bleeding history or a known bleeding disorder were excluded.

Interventional cardiac catheterization and follow-up

During ICC, children received a bolus of 100 IU/kg unfractionated heparin intravenously after establishing femoral vascular access, followed by a second bolus of 50 IU/kg after 60 min. After ICC, children received one dose of low molecular weight heparin subcutaneously at a dose of 1 mg/kg and aspirin was started the evening after ICC with a single daily dose of 2–5 mg/kg orally, rounded to the nearest half-tablet size. Aspirin therapy was continued for a total of 3 months after closure of patent arterial duct or balloon dilatation of valvular stenosis, and for 6 months in all other cases. Routine follow-up visits were carried out 1, 3, and 6 months after ICC and included physical examination, electrocardiogram, pulse oxymetry, and two-dimensional transthoracic echocardiography. Compliance for aspirin intake, bleeding events, and side effects were assessed by structured interview and questionnaire. Laboratory response to aspirin therapy was

measured 4–6 weeks after closure of patent arterial duct or balloon dilatation of valvular stenosis and 10–12 weeks after all other procedures.

Blood sampling and laboratory investigations

Peripheral blood samples were collected by venepuncture prior to cardiac catheterization, and 4–6 or 10–12 weeks after the procedure during a regular follow-up visit at the cardiac outpatient clinic. In children showing AR by arachidonate-induced aggregation (AA), the aspirin dose was increased to 6–10 mg/kg/day and additional testing was performed 1–3 weeks after dose increase.

Ten to 12 mL venous blood was collected into tubes containing 0.106 or 0.129 M (for PFA-100) sodium citrate as anti-coagulant (S-monovettes, Sarstedt, Nürnberg Germany); for blood cell counts, EDTA anti-coagulant was used. The samples were kept at room temperature and studied within 4 h of collection. Additionally, 5–10 mL urine was collected at the time of each blood sampling.

Platelet aggregometry Platelet aggregation, which evaluates responsiveness to different platelet-activating agents, e.g., AA and ADP, by measuring changes in light transmission in rapidly stirred citrated platelet-rich plasma, was performed with a four-channel aggregometer (APACT 4004, LABuTec, Ahrensburg, Germany). According to platelet aggregometry, AR was defined as >20 % aggregation response to 0.5 mM arachidonate and >70 % response to 10 μM ADP. AA has demonstrated a high and specific sensitivity to aspirin and is used as the gold standard for the determination of aspirin responsiveness in this and in other adult studies [12, 17, 31].

Urinary 11-dhTxB2 Production of this urinary thromboxane metabolite is suppressed by the inhibition of thromboxane synthesis by aspirin. Levels were measured using a quantitative enzyme-linked immunoassay kit (AspirinWorks1, Corgenix, Inc., Broomfield, CO) at the Hamilton Haemostasis Reference Laboratory, Hamilton, ON, Canada). Urine specimens were frozen within 2–4 h of collection at −70 °C for storage and were shipped with dry ice. AR was defined in comparison with urinary 11-dhTxB2 baseline levels: On aspirin, a level above the 10th percentile of age-correlated urinary 11-dhTxB2 excretion at baseline was considered as AR.

PFA-100® PFA-100 closure times (CTs) with the collagen/epinephrine (Col/Epi) cartridge are prolonged after ingestion of aspirin in the majority of patients. CT measurements were performed using citrated whole blood according to the manufacturer's instructions (Siemens Healthcare Diagnostics Inc., Erlangen, Germany). A normal Col/Epi CT (<164 s) during aspirin therapy was considered as AR [4].

Impact-R This cone and plate analyzer determines platelet adhesion to a polystyrene surface by a photodigital sensor under laminar flow. The manufacturer has developed a method to detect platelet inhibition by aspirin and has validated this method in adults. Analysis with the Impact-R® (DANED SA, Beersel, Belgium) was performed according to the manufacturer's instructions: The percentage of surface covered (% SC) provides information about platelet adhesion and aggregation. Aspirin response was investigated after addition of 0.275 μM arachidonate as the agonist, and AR was documented by a reduced SC to ≤3.4 % [33, 34].

Statistical analyses

Data are presented as means±SD for normally distributed data and otherwise, as median and interquartile range (IR). Student's *t* test and the Wilcoxon test were used to compare continuous variables between two groups. The Fisher exact test was used for comparing categorical data. Correlation was calculated using Spearman's correlation coefficient. Receiver operator characteristic (ROC) analysis was used to determine the sensitivity and specificity of the laboratory assays used. The agreement between the aspirin response assessed by AA and the other tests was evaluated using Cohen's kappa coefficient [2]. *P* values<0.05 were considered significant. Prism version 4.0 and QuickCalcs (GraphPad Software, CA, USA) and SPSS version 15.0 (SPSS, Zurich, Switzerland) were used for data analysis.

Results

Patient population

Of 150 children for whom informed consent was obtained, 45 were excluded for the following reasons: diagnosis of von Willbrand disease (VWD) (n=1) and history of relevant previous bleeding episodes obtained only after inclusion (n=2); use of another medication interfering with platelet function (n=5); poor venous access (n=3); consent withdrawal during the study (n=2); decision by the cardiologist to cancel the ICC (n=3), to perform open heart surgery (n=3), not to give aspirin (n=2), or to administer clopidogrel (n=1); and lost to follow-up (n=20). Three children were excluded, as venous (n=2) or arterial (n=1) femoral thrombosis occurred during the ICC procedure requiring low molecular weight heparin therapy after ICC. Interventional cardiac catheterization was

performed on the remaining 105 children; for diagnoses for ICC, see Table 1.

Clinical outcomes

Occasional non-compliance with aspirin intake was reported by 25 (23.8 %) of the 105 children, who forgot aspirin for 1 (n=11), 2–3 (n=5), 4–7 (n=2), or for "several days" (n=7). However, none of the patients with AR according to AA reported any non-compliance. Mean baseline aspirin dose per kilogram body weight did not differ significantly between children with normal aspirin response and AR (Table 1). During aspirin therapy and at any follow-up after ICC, none of the study subjects showed clinical or echocardiographic signs of thromboembolism at the occlusion or stent insertion site. Minor bleeding events were reported in 20 (19 %) children, including mild epistaxis in 12 children (11.4 %), of whom one had cauterization of locus Kisselbachii, two stopped aspirin intake for 3 days, and in one, the aspirin dose was reduced by 25 %. In eight children (7.6 %), easy bruising, unusual hematoma, and prolonged wound bleeding were reported. The median age of children with minor bleeding symptoms during aspirin was significantly lower (4.2 years) than that of children without bleeding (8.8 years; p=0.014). In three out of five children who had an aspirin dose increase (see below), minor bleeding events occurred only after this increase.

Laboratory results

Blood samples on aspirin were collected after a mean (median) follow-up time of 4.8 (5.1) weeks for children after patent arterial duct closure or balloon dilatation of valvular stenosis, and 14.6 (13.8) weeks in all other cases. Routine laboratory results at baseline, for all children, children with AR by AA, and children with AR identified by PFA-100 CTs, are presented in Table 1.

Platelet aggregation At baseline, 1 % of children showed abnormal response to AA (<80 % aggregation), and 11 % to ADP (<70 % aggregation). During aspirin therapy, AR was detected in 7 (6.7 %) children according to AA and in 61 (58 %) according to aggregation induced with 10 μM ADP (Table 2). Only one of the seven children with AR according to AA also had AR according to ADP-induced aggregation. After aspirin dose increase to the five available children with AR according to AA, four now demonstrated a normal response to aspirin, while only one child still had AR, documented by an AA aggregation of 95 % (the other two children with AR according to AA were unavailable for a dose increase or for additional follow-up testing).

Urinary 11-dhTxB2 Levels were measured at baseline (n=105) and during aspirin treatment (n=54). As higher urinary 11-dhTxB2 levels were found in younger children, age-specific levels for normal urinary 11-dhTxB2 excretion were

Table 1 Clinical and laboratory characteristics at baseline for all patients, for aspirin "resistant" patients (AR) according to arachidonate-induced aggregation (AA), and according to PFA-100 collagen-epinephrine closure times (PFA)

	All children (n=105)	Children with AR according to AA (n=7, 6.7 %)	Children with AR according to PFA (n=16, 15.2 %)
Median age (years, range)	7.6 (0.1–19.8)	5.3 (4.8–16.5)	6.8 (2.8–15.7)
Sex (M:F)	49:56	2:5	5:11
Diagnosis for ICC (n)			
ASD	36	2	11
VSD	7	–	–
PAD	20	–	3
Vascular malformation of pulmonary artery (stent implantation)	11	2	1
Coarctation of aortic arch	18	2	1
Other vascular	2	–	–
malformation		–	–
Aortal valve stenosis	3	–	–
Pulmonary valve stenosis	8	1	–
Mean aspirin dose (mg/kg/day)	3.2±1.2	2.9±0.8	3.3±1.2
Hematocrit (%)	38±4	38±2	39±4
Platelet count (×10^9/L)	285±61	298±46	282±39
INR	1.08±0.1	1.08±0.06	1.07±0.06
PTT (s)	35.7±4.4	34.3±2.7	35.3±2.8
VWF:RCo (%)	94.5±20.7	99.7±23.6	104.4±19.2

Results are presented as mean± SD if not stated otherwise. Student's t test results are given for statistically significant differences (p<0.05) only

ICC interventional cardiac catheterization, *ASD* atrial-septal defect, *VSD* ventricular-septal defect, *PAD* patent arterial duct, *VWF:RCo* von Willebrand factor:ristocetin cofactor

Table 2 Sensitivity specificity and agreement of tests for the evaluation of aspirin response in children

	Abnormal result at baseline	Aspirin "resistance"	ROC (AUC)[a]	Kappa compared to AA[b]
AA (0.5 mM)	1 %	6.7 %[c]	0.98	–
Urinary 11-dhTxB2	na	12.9 %[c]	0.92	0.673
PFA-100 Col/Epi CT	29.5 %	15.2 %[d]	0.86	0.008
ADP-induced aggregation	11 %	58 %[d]	0.72	0.001
Impact-R SC	31.6 %	6.3 %[d]	0.55	0.092

na not available

[a] Receiver operator characteristic (ROC) analysis: area under the curve (AUC)

[b] Cohen's kappa coefficient of agreement (kappa): agreement in comparison with arachidonate-induced aggregation (AA). The strength of agreement is interpreted as follows: <0.20 poor; 0.21–0.60 fair–moderate; 0.61–0.80 good; >0.81–1 very good [2]

[c] Significant correlations were found between results from AA and urinary 11-dehydro-thromboxane B_2 (urinary 11-dhTxB2) levels (r=0.45; P < *0.001*)

[d] No correlations were seen between AA and all other laboratory methods (PFA-100 collagen/epinephrine closure times, PFA-100 Col/Epi CT; ADP-induced aggregation; and Impact-R surface coverage, Impact-R SC) (r<0.1; P>0.1)

established (Fig. 1). Before aspirin intake, the median urinary 11-dhTxB2 (ng/mmol creatinine) was 517.5 (IR 407-753; n=8) in children under 2 years, 297 (IR 205-363; n=37) for 2–<6 years, 221 (IR 179-268; n=24) for 6 to 12 years, and 146 (IR 96.5-187.8; n=36) in children over 12 years of age (P<0.001 for all age groups). During aspirin therapy, median urinary 11-dhTxB2 decreased significantly (P<0.001). A comparison of results at baseline and during aspirin therapy for each age group is presented in Fig. 1. AR, defined as urinary 11-dhTxB2 levels on aspirin above the 10th percentile of baseline levels, was found in seven (12.9 %) children. Comparing AA and urinary 11-dhTxB2 excretion, five of the seven children with AR according to urinary 11-dhTxB2 also had AR according to AA (Fig. 1).

PFA-100 CTs At baseline, prolonged Col/Epi CTs were observed in 29.5 % of children. During aspirin therapy, a normal response to aspirin with a prolonged CT was present in 89 (84.8 %) children and AR was detected in 16 (15.2 %) children. Of these, only one also had AR according to AA. In seven children with AR according to AA, only one also had AR according to Col/Epi CTs. Children showing prolonged CTs at baseline demonstrated AR in only 1 out of 16 subjects according to PFA-Col/Epi CTs but tended to have decreased VWF:RCo levels (85.9±20.3 %, n=31, compared to those with baseline CTs in the normal range (95.9±25.7 %, n=74; P=0.09.). During aspirin therapy, in the 16 children with normal Col/Epi CTs, indicating AR, significantly increased von Willebrand factor:ristocetin cofactor (VWF:RCo) levels (118 ±32.6 %, n=16) were observed in comparison with children with normal aspirin response and prolonged CTs (94.7± 25.7 %, n=89; P<0.01).

Impact-R SC These determinations were done on 95 children. At baseline, before the start of aspirin therapy, mean SC was 10.1±5.4 % in the absence of agonist. Unexpectedly, added arachidonate did not significantly decrease the mean SC (8.5±

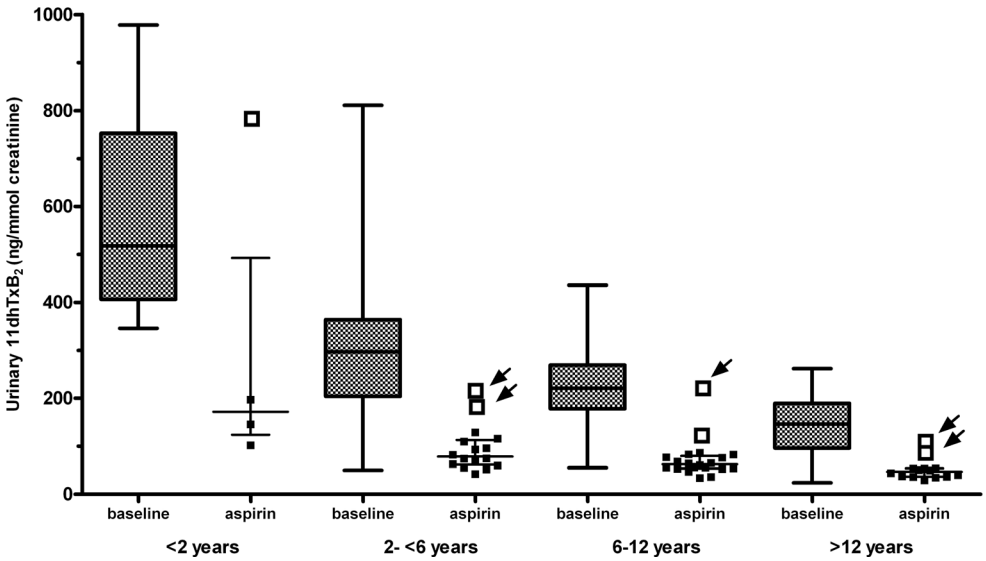

Fig. 1 Urinary 11-dehydro-thromboxane B_2 (11-dhTxB2) levels. Results at baseline before the start of aspirin (*box* and *whiskers*: children<2 years, n=8; 2–<6 years, n=37; 6–12 years, n=24; and >12 years, n=36) and during aspirin treatment (*dot plot*, median and interquartile range, children<2 years, n=4; 2–<6 years, n=16; 6–12 years, n=20; and >12 years, n=14). *Open squares* indicate children with aspirin "resistance" according to urinary 11-dhTxB2 (i.e., results above the 10th percentile of baseline during aspirin treatment). The *arrows* identify five children who also demonstrated aspirin resistance according to arachidonate-induced aggregation

4.1 %; $P>0.1$), and during aspirin therapy, the mean SC with added arachidonate was similar (8.9±3.5 %) to baseline. On aspirin, six patients (6.3 %) had SC results ≤3.4 %, indicating AR. Of these, only one had a baseline SC ≤3.4 %, and only one had AR according to AA and one according to the PFA-100 Col/Epi CT.

Receiver operating characteristic (ROC) curve analysis was utilized to assess the diagnostic performance of all five tests (Fig. 2). AA and urinary 11-dhTxB2 levels showed high sensitivity and specificity for aspirin (ROC area under the curve (AUC) 0.98 and 0.92, respectively), while the ROC AUCs were lower for PFA-100 Col/Epi CTs (0.86), aggregation induced with 10 μM ADP (0.72), and Impact-R % SC (0.55), indicating lower sensitivity and specificity for these tests. By the use of Cohen's kappa coefficient of agreement, AA and urinary 11-dhTxB2 levels demonstrated good agreement between test results (Table 2).

Discussion

For children with congenital heart disease, aspirin is often administered for the prevention of thromboembolism following catheter interventions or surgical procedures. Only small and uncontrolled studies on the dose response to and clinical outcome from aspirin therapy in children have been published. In the absence of large prospective trials, cardiologists mostly manage pediatric patients according to published guidelines for adults [3, 23, 26, 36].

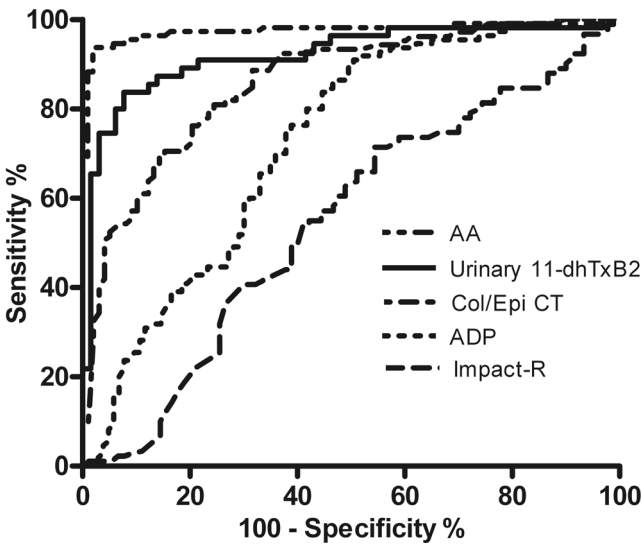

Fig. 2 Receiver operator characteristic (ROC) curves. Results for 0.5 mM arachidonate-induced aggregation (*AA*), urinary 11-dehydro-thromboxane B_2 levels (*Urinary 11-dhTxB2*), PFA-100 collagen/epinephrine cartridge closure times (*Col/Epi CT*), aggregation induced by 10 μM ADP (*ADP*), and Impact-R % surface coverage (*Impact-R*)

The present study was performed in a large and well-defined cohort of 105 pediatric patients. Following ICC, children were treated with a standardized dose of 2–5 mg/kg orally. Using the "gold standard" test (AA), AR was detected in 6.7 %. In the majority of children with AR and who were available for follow-up, an aspirin dose increase to 6–10 mg/kg resulted in the expected inhibition of AA. Aspirin therapy was safe and our data might indicate that aspirin is effective in preventing pediatric ICC patients from thromboembolism, although patients not given aspirin were not studied. No difference in the ages of patients with and without AR were found (Table 1). The influence of food ingestions and diet was not studied in our cohort; recently, however, a reduced bioavailability of aspirin due to high milk intake by adults was observed [27].

In our study, no major bleeding event (i.e., gastrointestinal or intracranial bleeding or bleeding requiring transfusion) was observed. Still, 19 % of children had minor bleeding symptoms, and of those, the median age was significantly lower than that of children without bleeding. In the seven children after aspirin dose increase, more than half had epistaxis and bruising. Due to the short follow-up time and small number, it is difficult to draw conclusions from this; however, some adult studies indicate that more severe bleeding events are seen in patients on higher aspirin doses [32].

The strength of our study is that the anti-platelet activity of aspirin was determined by different platelet function tests. The aim was to evaluate the usefulness of tests commonly used in children for the determination of aspirin response. While results from AA, the gold standard test, indicated a prevalence of AR similar to adults, all other tests, i.e., urinary 11-dhTxB2 excretion, aggregation induced by 10 μM ADP, and the PFA-100 CTs, showed AR at a higher frequency but, except for urinary 11-dhTxB2 levels, also demonstrated an inferior specificity and/or sensitivity for aspirin. Our results are in keeping with those from studies in adults, indicating a poor agreement in between tests [5, 24, 31]. The Impact-R test, which is mainly used for research purposes, has been used to measure aspirin response. We found that it demonstrated a high degree of abnormal results at baseline, as also found by others, and a weak concordance with the other tests [11].

Urinary 11-dhTxB2 levels are a commonly used measure of thromboxane synthesis, which is suppressed by aspirin. They have been validated as a predictive determinant of stroke, myocardial infarction, or cardiovascular death in adults [7, 8]. Results of this and two other studies have found higher urinary 11-dhTxB2 levels in younger children [38, 40]. Thus, we found it necessary to establish age-specific baseline reference values to subsequently define AR. Still, and in contrast to all other tests, a good agreement and a moderate, but significant, correlation was observed between urinary 11-dhTxB2 excretion and AA ($r=0.45$, $P<0.001$) (Table 2). Of the seven children on aspirin that showed high urinary 11-dhTxB2

excretion above the 10th percentile of baseline levels, five also demonstrated AR according to AA. The determination of urinary 11-dhTxB2 excretion performed with small urine samples appears to be an elegant way of testing aspirin response in children; however, previous studies demonstrated that this test is not specific for platelet cyclo-oxygenase activity. Thromboxane A_2 production by monocytes, erythrocytes, and endothelial or renal cells has been described in both children and adults; increased levels of urinary 11-dhTxB2 could also reflect the presence of systemic disease such as severe pulmonary disease, cyanosis, renal disease, or infections and would interfere with the assessment of the platelet thromboxane synthesis [1, 20, 41].

In our study, AR was detected in 15.2 % of children by PFA-100 CTs. Many previous studies have investigated AR using PFA-100 Col/Epi CTs, as these are prolonged by aspirin [11, 14, 29, 40]. The PFA-100 is a popular test in many pediatric centers given its simple use and requirement of only a small volume of blood for testing; it can detect VWD or abnormal platelet function. With this test, two pediatric studies showed quite discordant prevalence rates of AR, specifically 12 and 26 % [16, 40]. Only one of the studies compared the PFA-100 results with AA: While no correlation was found between the tests, AR determined by the PFA-100 was found in 12 % and in 5 % by AA. In contrast to our study, that study used a variety of and less standardized doses in their cohort (0.9–8 mg/kg) and only evaluated a minority of their patients off and on aspirin [40].

In the present study, we unexpectedly observed a high rate of abnormal PFA-100 results at baseline. These prolonged CTs that were seen in the light of normal platelet counts, hematocrit, and VWF levels (Table 1) can only partially be explained by the lower cut off for normal pediatric Col/Epi CTs (<164 s) that we used compared with other studies [4, 16, 40]. Although only patients with a negative bleeding history were included in our study, the presence of patients with mild defects of primary hemostasis cannot be excluded, as mild platelet dysfunction can be present with very subtle symptoms and many of the young children had surgical interventions for the first time [28]. In comparison, only 1 % of children had abnormal results with the thromboxane A_2-specific AA at baseline, while the Impact-R, like the PFA-100, showed a significant number of abnormal results (31.6 %) at baseline (Table 2). At baseline, also 23.8 % had prolonged CTs with both Col/Epi and Col/ADP CTs (data not shown). The increased rate of abnormal CTs was not investigated further and other factors (e.g., the problem of standardization of tourniquet use in children during difficult blood sampling procedures) might be contributory [37].

PFA-100 CTs have been shown to be highly dependent on levels of plasma VWF that could also influence the prevalence of AR according to the PFA-100 CTs [9, 21]. Our results confirm that children with AR according to PFA-100 CTs have significantly increased VWF:RCo levels compared to children without AR. Data from studies in adults indicate that VWF can be increased due to physical and mental stress, which could occur in infants and children during the blood sampling procedure [18, 39]. All the above mentioned findings could make it difficult to diagnose AR in individual children by the use of the PFA-100 CTs alone.

This study has several limitations. As aspirin treatment is the standard of care in our institution, we were unable to include controls that did not receive aspirin after ICC. So, as we did not observe any thromboembolic events during our follow-up period, it is unclear if in children after ICC, aspirin is required at all. This needs to be studied in future randomized controlled studies. In addition, follow-up urine samples were available in 51 % of our patients after ICC, which allows us to give only approximate data about the agreement of AA and urinary 11-dhTxB2 excretion.

In conclusion, the results of this pediatric study comparing different laboratory tests show an AR prevalence of 6.7 % according to AA, which is similar to that seen in recent adult studies [12, 13]. In the majority of cases, AR could be overcome by an increase in the aspirin dose. The most specific test for detecting AR is AA, while PFA-100 CTs and Impact-R SC results have a lower sensitivity and specificity, and urinary 11-dhTxB2 excretion can be biased by concurrent clinical conditions or thromboxane production by cells other than platelets. In this study, an aspirin dose of 2–5 mg/kg was effective in inhibiting platelet function in the majority of children. Still, it is unknown if aspirin is required after ICC at all. This has to be taken into account as a significant number of children demonstrated bleeding symptoms on aspirin; although no major bleeding events occurred, minor bleeding might be even more frequent in younger children and when doses >5 mg/kg are applied. In line with current recommendations for adults, testing for aspirin response does not appear to be generally indicated in pediatric patients [25]. Most importantly, pediatricians should enforce compliance of aspirin intake. However, testing for aspirin response could be applied in individual cases, particularly when the thromboembolic risk requires a profound and well-documented inhibition of platelet thromboxane synthesis, when a possible interaction with food or concomitant medication needs to be monitored, or when the treating physician wants to apply an aspirin dose in the lower range, i.e., in order to minimize the bleeding risk.

Conflict of interest The authors declare that they do not have any conflict of interest.

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